REMARKS

Claims 1-34 are pending with claims 12-13 withdrawn. The amendment to claims 1 and 2 is supported in the specification at p.8, line 14 among other places. No new matter has been added.

IDS

A corrected IDS has been paper filed resubmitting legible copies of each cited foreign patent document. This should address this issue.

Rejection under 35 USC § 102(b)

Claims 1-11 are rejected under 35 U.S.C. 102(b) as being anticipated by Nishibu et al. (Analytical Biochemistry, vol. 319, pp. 88-95 (2003)) ("Nishibu"). (Office Action page 3)

Attached is an unequivocal declaration under 37 CFR § 1.132 from T. Nishibu, K.Hirayasu and Y.Kobayashi that they conceived the subject matter disclosed in the article and relied on in the rejection. This is sufficient for showing that the reference invention is not "by another." (MPEP §§ 715.01(c); 716.10)

The 35 USC § 102(b) rejection is overcome based on the fact that the reference is not "by another" and that the PCT application was filed within one year after the publication of the article.

Rejection under 35 USC § 103

The claimed invention of amended claim 1 is

> A method for immobilizing a protein to a solid-phase, comprising filtrating the protein with the solid-phase having hydrophobic surface in the presence of a lower alcohol, and a halogenocarboxylic acid and/or a long chain alkyl sulfate.

A biochemical sample generally contains many substances other than the protein such as a surfactant (present specification, p.1, lines 24 to 26). However, since a protein in a sample in the

presence of a surfactant cannot be immobilized efficiently by the conventional immobilization method, quantitative determination of a protein in such a sample cannot be achieved, but according to the claimed invention, a protein in such a sample can be immobilized efficiently (p.8 to p.9).

Generally, surfactants such as anionic surfactant (ex. SDS) have a property to inhibit adsorption of a hydrophobic substance. On the contrary, binding of a protein to a membrane is thought to occur by hydrophobic bonding. For this reason, surfactants have not been preferably used in the immobilization of proteins. Consequently, there are few reports discussing an action of a surfactant against a protein and an interaction between the protein receiving the action and a solid-phase surface in detail. For this reason, it has not been known until now that a long chain alkyl sulfate as a kind of surfactant is effective for immobilization of a protein (p.8, lines 20-30).

Accordingly, the idea, which a protein can be sufficiently immobilized to a solid-phase in the presence of a long chain alkyl sulfate together with a lower alcohol, or together with a lower alcohol and a halogenocarboxylic acid, is found by the present inventors for the first time (p.8) Therefore, it is one of an advantage in the claimed invention that a protein in a sample containing surfactant such as SDS can be immobilized efficiently.

Claims 1-5 and 8-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheley et al. (Biotechniques, vol. 10, no. 6, pp. 731-732 (1991), cited in the IDS filed July 22, 2008) ("Cheley") in view of Jacobson (Electrophoresis, vol. 11, pp. 46-52 (1990), cited in the IDS filed July 13, 2006). (Office Action page 5, 6)

First, claims 1 and 2 have been amended to distinguish the invention over the art. The invention disclosed in Jacobson is to transfer protein from gel after electrophoresis to nitrocellulose membrane. In Jacobson, "two membranes of the same type were placed on top of the gel and on each side of the package 3 filter papers were positioned." On the other hand, the present claim 1 is "A method for immobilizing a protein to a solid-phase, comprising filtrating the protein with the solid-phase..." The procedure of "filtrating a sample" does not include the meaning of simply "be placed on a sample," thus the two methods are functionally distinct, among other differences, as will be explained herein.

While Office Action alleges that Cheley teaches a method of immobilizing protein

samples to a nitrocellulose membrane using a dot blotter, in which the samples are mixed with a solution of SDS and TCA; Cheley *does not teach that a lower alcohol is used with the halogenocarboxylic acid and long chain alkyl surface.*

In contrast, the claimed invention is a method for immobilizing protein on the membrane using lower alcohol and SDS and/or TCA. That is, lower alcohol is an *essential component* of the present invention (see claim 1).

As is clear from Fig.1 and the description in the specification on p.33 to 34:

> When the immobilization sample 2 containing SDS and TCA (which does not contain alcohol) was used (This is the same condition as the method disclosed in Cheley.), the signal intensity was slightly increased (measurable), but the signal intensity as high as the control (in the case using purified water, that is, conventional immobilization method) could not be measured.
>
> Contrary, when the immobilization sample containing ethanol and SDS and/or TCA (immobilization samples 3 to 5) were used, the signal intensity equivalent to or higher than the control could be measured. ...
>
> From the above facts, it is understood that the immobilization rate of a protein to a membrane can be improved spectacularly by the immobilization method of the present invention which is conducted in the presence of a lower alcohol, a halogenocarboxylic acid and/or a long chain alkyl sulfate, as compared with the conventional immobilization method using only water or a burrer solution.

The invention disclosed in Jacobson is to *transfer protein from gel* after electrophoresis to nitrocellulose membrane, but is *not to immobilize the protein on the membrane.*

For instance, in p.47, left column "2.2 Transfer", Semi-dry electrophoretic transfer was performed. That is, two membranes of the same type were placed on top of the gel and on each side of the package 3 filter papers were positioned. The transfer was performed at a current of 12.5 am/gel (0.8mA/cm2) which resulted in a voltage of 5-7V.

As is clear from Figures 1-a) to b) and disclosure of p.47, left column, "3 Results, 3.1 Buffer composition," soybean trypsin inhibitor and BSA were eluted from the gel in 20-30 min. Maximum (85-95%) binding of soybean trypsin inhibitor and BSA to the first membrane was

obtained after 20-30 min. If the transfer was continued longer, the proteins leaked from the first membrane (p.47, left column, bottom line to p.47, right column, line 2). And, the transfer time is passed, proteins migrate from gel—> Nitrocellulose 1—>Nitrocellulose 2—>Filter paper, and therefore, it shows that the *protein can not be immobilized on the membrane* well (Figs. 1 to 3) by Jacobson's method.

Additionally, Jacobson disclose that "the binding to nitrocellulose was lower in the presence of SDS" (Jacobson, p.47, right column, lines 17 to 18, Fig.2b), and "The addition of 0.1% SDS to the transfer buffer may result in improved elution efficiency of high molecular weight proteins, but, on the other hand, SDS reduces the binding to nitrocellulose" (p.49, right column, lines 14 to 17).

In contrast, a protein can be immobilized by the immobilization method of the present invention even when the sample containing SDS in advance is used. For instance, the signal intensity equivalent to or higher than the control could be measured by using the immobilization samples 3 (0.19% (W/V) SDS, 42.2% (V/V) ethanol) and 4(0.19% (W/V) SDS, 2.34% (W/V) TCA, 42.2% (V/V) ethanol) (present specification p.32).

The Office Action asserts that it would have been obvious to one of ordinary skill in the art to modify Cheley's method to include a PVDF membrane and methanol in the transfer buffer, as taught by Jacobson.

However, as is clear from above, "the method of Cheley which is immobilizing the protein on the membrane by filtrating the sample using a dot blotter" and "the method of Jacobson which is transferring the protein from gel to membrane at a current of 12.5mAigel" are ***different in the principle of immobilization and measurement procedures.*** Additionally, though the immobilization of protein on the nitrocellulose membrane can not be performed well by Jacobson's method, Cheley's method is to immobilize protein on the membrane. Further, though *SDS is not preferable for use in Jacobson*, SDS is essentially used in Cheley.

Therefore, there is no motivation to combine Jacobson with Cheley.

Further, as mentioned above, a protein can be immobilized by the immobilization method of the present invention even when the sample containing SDS in advance is used. ***This important feature of the claimed invention is neither disclosed nor suggested by the cited references***, and therefore, this advantageous effect of the present invention is totally unexpected by those skilled in the art.

It is respectfully requested that the rejection be reconsidered and withdrawn.

Claims 6-7 and 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cheley in view of Jacobson, both cited and described above, directed to claim 1. (Office Action page 8)

For the same reasons explained above, there is no motivation to combine Jacobson with Cheley, and the claimed invention has the above-described unexpected superior effect to the disclosure of Cheley and Jacobson.

It is respectfully requested that this rejection be reconsidered and withdrawn.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105.

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Respectfully submitted,

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Encls: Declaration under 37 CFR § 1.132